AMENDMENTS

In the specification:

Please amend the specification to include SEQ ID NO: as follows:

On Page 3, please delete the paragraph on Page 3, line 22, to page 4, line 13, and substitute therefor:

Figure 1 shows a schematic representation of plasmid DNA γ1WT and its γ1WT-TAC and γ1NANP variants. The γ1WT H chain construct is the product of the fusion between a human γ1 constant (C) region gene present in the plasmid vector pNeoγ1 with the murine V_H62 gene (2.3 kb) (Sollazzo et al., *supra* , 1989). The V_H region gene is productively rearranged and the C region gene is in genomic configuration. Variants γ1WT-TAC and γ1NANP contain the nucleotide insertions shown in bold characters in CDR3 (γ_1WT, SEQ ID NO:1; γ_1WT-TAC, SEQ ID NO:2; γ_1NAN, SEQ ID NO:3). Each plasmid DNA carries the regulatory elements, promoter (Pr) and enhancer (En) needed for tissue-specific expression. In plasmid DNA γ1NANP the humanγ1 C region gene is joined to a productively rearranged murine variable (V) region gene modified in the third complementarity determining region (CDR3) by introduction of the nucleotide sequence coding for three Asn-Ala-Asn-Pro repeats (SEQ ID NO:4). In these plasmids, the promoter and enhancer elements are those constitutively existing in Ig H chain genes. Neo^r=neomycin resistance gene; Amp^r=ampicillin resistance gene; PR=promoter; EN=enhancer; C_H=heavy chain C region; V_H=heavy chain variable region; FR=framework region; CDR=complementarity determining region.

On Page 4, please delete the paragraph on Page 4, line 14, to Page 4, line 29, and substitute therefor:

Figure 2 shows the nucleotide sequence of genomic DNA clones corresponding to the productively rearranged VDJ region of γ1WT-TAC DNA. A 520 bp fragment was amplified from (1) genomic DNA extracted from a spleen inoculated 17 days earlier with plasmid DNA γ1WT-TAC, and (2) J558L cells constitutively harboring plasmid DNA γ1WT (Sollazzo et al., *supra* , 1989). The amplified products were cloned and sequenced using two different primers from opposite directions. The top nucleotide sequence refers to γ1WT-TAC (SEQ ID NO:5) and serves as a reference. SP7-SP12 identify six clones originated from splenic genomic DNA (SP7, SEQ ID NO:6). TR35-TR38 identify four genomic DNA clones derived from transfectoma cells (TR35, SEQ ID NO:7; TR38, SEQ ID NO:8). The CDR and framework regions (FR) are indicated. This study indicates that after injection *in vivo* the transgene does not undergo somatic mutation.

On Page 5, please delete the paragraph on Page 5, line 21 to Page 6, line 12, and substitute therefor:

Figure 5 shows engineering and expression of an immunoglobulin H chain gene with two heterologous epitopes. Panel A shows a schematic representation of the mutagenesis vectors, introduction of the (NANP)3 and NANPNVDPNANP (SEQ ID NO:9) coding sequences and partial, nucleotide sequence of CDR2 (SEQ ID NO:10) and CDR3 (SEQ ID NO:11) after insertion. The synthetic oligonucleotides and the mutagenesis steps for the creation of pVH-TAC/CCA are detailed in the Experimental Protocol. Two pairs of complementary synthetic oligonucleotides coding for (NANP)3 and NANPNVDPNANP, were cloned in the Asp718 site in CDR3 and in the NcoI site in CDR2 of pVH-TAC/CCA. The insertions were verified by dideoxy- chain-termination sequencing. Panel B shows a schematic representation of plasmid DNA γ1NV^{2}NA^3 carrying the coding sequences for the two heterologous epitopes in CDR3 and CDR2, respectively. The human γ1 constant (C) region gene is in genomic configuration. CH1, CH2, and CH3 refers to the corresponding domains in the C region of the

γ1 gene. Promoter (Pr) and enhancer (En) elements for tissue-specific expression and the neomycin (Neo^r) and ampicillin (Amp^r) resistance genes are indicated. Panel C shows a schematic representation of antigenized H chain gene product paired with a light chain. The engineered epitopes in CDR3 and CDR2 are as indicated (not to scale).

On Page 8, please delete the paragraph on Page 8, line 29 to Page 9, line 20, and substitute therefor:

Figure 13 shows a schematic representation of plasmid DNA γ1NP. This H-chain coding plasmid is the product of the fusion of a human γ1C region with a murine VH engineered to express the 13 amino acid residues from the sequence of the influenza virus nucleoprotein (NP) antigen (366-379) in the third complementarity-determining region (CDR3) (SEQ ID NO:12). This NP peptide is presented in association with the Db allele in H-2b mice. The coding strand of the CDR3 region is shown in bold, with the NP-coding sequence underlined. The amino acid sequence of the influenza peptide 366ASNENMETMESSTL379 (SEQ ID NO:13) is shown in bold. B, BamHI; RI, EcoRI; Neo, neomycin (G418) resistance; Amp, ampicillin resistance. The H-chain gene was mutagenized to introduce a single KpnI/Asp718 site and complementary oligonucleotides 5' GTA CCC GCT TCC AAT GAA AAT ATG GAG ACT ATG GAA TCA AGT ACA CTT 3' (SEQ ID NO:14), 5' GTA CAA GTG TAC TTG ATT CCA TAG TCT CCA TAT TTT CAT TGG AAG CGG 3' (SEQ ID NO:42) coding for residues 366-379 of the influenza virus NP antigen (ASNENMETMESSTL) were introduced between 94V and 95P of the mutagenized VH region. The engineered VHNP coded by the 2.3 kb EcoRI fragments was cloned upstream from a human γ1 constant (C) region gene contained in the 12.8 kb vector pN γ1.

On Page 10, please delete the paragraph on Page 10, line 22 to Page 11, line 7, and substitute therefor:

Figure 36 shows the effect of linked recognition of a dominant Th epitope and a cryptic/subdominant Th epitope on the proliferative response to the cryptic/subdominant epitope. Th/Th associative recognition is necessary to render immunogenic T cell determinant from the

MUC-1 antigen. Mice were inoculated with plasmid DNA as indicated. Spleen cells were harvested on day 15 and re-stimulated *in vitro* for 4 days in the presence of 50 μg/ml of synthetic peptide (DTRP)3 (SEQ ID NO:16) and VTSAPDTRPAP (denoted as VTSA). Both sequences are contained in the PDTRPAPGSTAP (SEQ ID NO:17) tandem repeat of the tumor antigen MUC-1. Superscript numbers indicate the CDR in which the heterologous antigen sequence has been inserted. Subscript numbers indicate the number of times the sequence in brackets is repeated in the context of a particular CDR. The results shown are cumulative of three independent experiments. Each group is constituted of 8-10 mice. Results are expressed as stimulation index. Bars indicate means of stimulation indexes ± SEM.

On Page 37, please delete the paragraph on Page 37, line 18 to Page 38, line 6, and substitute therefor:

For extraction of genomic DNA from spleen tissue and genomic DNA sequencing, spleens were harvested 17 days after DNA inoculation, frozen at -170°C and the cells were prepared by tissue grinding in liquid nitrogen. Typically the genomic DNA was extracted from 10 mg of spleen tissue using the QIAamp Tissue Kit (Qiagen Inc.; Chatsworth CA). Two specific primers, TTATTGAGAATAGAGGACATCTG (SEQ ID NO:22) and ATGCTCAGAAAACTCCATAAC (SEQ ID NO:23) for the murine V_H^{62} were used to amplify by PCR a segment of 520 bp from genomic DNA. The PCR conditions were as follows: 45 sec at 94°C, 45 sec at 54°C and 45 sec at 72°C for 30 times. The PCR products were cloned in pGEM-T vector (Promega; Madison WI). Six clones from the genomic DNA of the spleen inoculated 17 days earlier and four clones from the genomic DNA of tranfectoma B cells (Sollazzo et al., *supra*, 1989) were sequenced on both strands by dideoxy termination method with Sequenase 2.0 DNA sequencing kit (USB; Cleveland OH) using two primers, AACAGTATTCTTTCTTTGCAGG (SEQ ID NO:24) and TTATTGAGAATAGAGGACATCTG (SEQ ID NO:22), annealing 10 bp before the first codon of the FR1 and at the 3' end of the FR4, respectively.

At Page 43, please delete the paragraph beginning at Page 43, line 18 to Page 44, line 25, and substitute therefor:

To demonstrate that B lymphocytes are the target cell population *in vivo* for the transgene, the following experiment was performed. Starting from the second week after plasmid DNA inoculation, splenic B and T lymphocytes were isolated to a high degree of purity (97-99%) by FACS sorting (Figure 3). The genomic DNA was extracted from the two cell populations and amplified by PCR. PCR was performed with a total of four sets of primers, pCL and pCD; pSE and pNAD; pNEL and pNED; and pγA1 and pγA2. pCL [[γ]]from -107nt to -85nt: 5'-TTATTGAGAATAGAGGACATCTG-3' (SEQ ID NO:22); and pCD [[γ]]from 459nt to 439nt: 5'-ATGCTCATAAAACTCCATAAC-3' (SEQ ID NO:25); were used to amplify the whole VDJ region of the transgene. pSE [[γ]]from -32nt to -11nt: 5'-AACAGTATTCTTTCTTTGCAGC-3' (SEQ ID NO:26); and pNAD [[γ]]from 352nt to 333nt: 5'-GAGAGTAGGGTACTGGGTTT-3' (SEQ ID NO:27); were specific for amplification of the genetic marker, $(NANP)_3$ in CDR3. pNEL [[γ]]from 169nt to 189nt: 5'-AGCACCTACTATCCAGACACT-3' (SEQ ID NO:28); and pNED [[γ]]from 366nt to 346nt: 5'-GTAGTCCATACCATGAGAGTA-3' (SEQ ID NO: 29); were the inner primers for nested PCR. pγA1 [[γ]]from 184nt to 201nt: 5'-TGGGCCGCCCTAGTCACC-3' (SEQ ID NO:SEQ ID NO:30); and pγA2 [[γ]](from 427nt to 408nt: 5'-CGTTTGGCCTTAGGGTTCAG-3' (SEQ ID NO:31); were designed to amplify the murine β-actin gene according to the sequence indicated in (Harris et al., Gene 112:265-266 (1992)). The PCR consisted of 30 cycles at 94°C for 45 sec, 58°C for 45 sec, and 72°C for 45 sec; 0.3 μM each primer; 0.2 mM each deoxynucleotide; 1.5 mM $MgCl_2$ in 20 mM Tris-HCl, pH 8.4 and 50 mM KCl; and 1 unit of T*aq* DNA polymerase (Gibco BRL; Gaithersburg MD). PCR products for Southern blot analysis were resolved in 1% w/v agarose gel and blotted onto HYBOND-N nylon membrane (Amersham; Cleveland, OH). The membranes were hybridized with the oligonucleotide pNAD labeled using T4 polynucleotide kinase forward reaction in presence of (γ^{32}P-ATP). At the 15 day time point, distinct amplification products were readily detectable in both B and T lymphocytes (Figure 3, left panel). However, at both the 21 and 28 day time points, specific amplification was observed

only in B cells (Figure 3, middle and right panels). Southern blot hybridization confirmed the specificity of the amplification products. These results suggested that B lymphocytes in the spleen are the target cell population in which the transgene persists for a long time.

On Page 48, please delete the paragraph beginning at Page 48, line 13 to Page 48, line 32, and substitute therefor:

Inoculation of plasmid γ1NANP DNA γ1NANP induces a primary response against the peptide NANP. Table 4 summarizes the ELISA antibody responses in which anti-NANP peptide antibodies were found in mice primed with the H chain transgene (γ1NANP DNA) (groups I and II). Antibodies appeared by day 14 and reached a plateau by day 28 (log 2.8)(Table 4). Circulating antibodies persisted through day 200 when mice received a booster injection. The antibody response against the intact antigenized antibody γ1NANP paralleled the response against the synthetic peptide. Mice inoculated intrasplenically with 50 μg of the γ1NANP protein (group IV) failed to mount any measurable anti-peptide response, although a modest elevation in titer against the intact γ1NANP antibody was measured. Control groups injected with either the pSVneo plasmid or with ovalbumin failed to develop any antibody response above background titers higher than the pre-immunization values. No binding was observed when the same sera were tested on the synthetic peptide DENGNYPLQC (SEQ ID NO:32) used as a control.

At Page 52, please delete the paragraph beginning at Page 52, line 3 to Page 53, line 9, and substitute therefor:

Plasmid $\gamma 1NV^{2}NA^{3}$ was engineered as described below. The EcoRI fragment of the productively rearranged murine VH (2.3 Kb) was cloned in vector pBluescript II KS to yield plasmid pVH. Site-directed mutagenesis was performed using two 21mer oligonucleotide primers, one (5'-CAAGAAAGG**TAC**CCTACTCTC-3') SEQ ID NO: 33) annealing in CDR3 to introduce 3bp (TAC, in bold) for the creation of an Asp718 site, and another (5'-

AGTAATGG**CCA**TGGTAGCACC-3') (SEQ ID NO:34) annealing in CDR2 to introduce 3bp (CCA, in bold) for the creation of a NcoI site. These primers were annealed to the uracylated, complementary strand of pVH and the mutant strands were synthsized and ligated in the presence of T4 DNA polymerase and ligase. Plasmid pVH-TAC/CCA, containing two unique sites, one in CDR3 (Asp718) and the other in CDR2 (NcoI), was obtained after transformation, screening of individual colonies and confirmation by DNA sequencing (SEQUENASE 2.0 DNA Sequencing Kit; USB; Cleveland OH). A pair of complementary oligonucleotides, 5'-GTACCCAATGCAAACCCAAATGCAAACCCAAATGCAAACCCA-3' (SEQ ID NO:35) (sense) and 5'-GTACTGGGTTTGCATTTGGGTTTGCATTTGGGTTTGCATTGG-3' (SEQ ID NO:36) (antisense) coding for the (NANP)3 sequence was synthesized, annealed and cloned in the Asp718 site. A pair of complementary oligonucleotides 5'-CATGGTAATGCAAACCCAAATGTAGATCCCAATGCCAACCCA-3' (SEQ ID NO:37) (sense) and 5'-CATGTGGGTTGGCATTGGGATCTACATTTGGGTTTGCATTAC-3' (SEQ ID NO: 38) (antisense) coding for the NANPNVDPNANP sequence was similarly cloned into the NcoI site. The insertions and the proper orientation were verified by dideoxy sequencing (SEQUENCASE 2.0 DNA Sequencing Kit; USB). The 2.3Kb EcoRI fragment carrying the engineered CDR3 and CDR2 was then subcloned in the expression vector pNÿ1 (Sollazzo et al., *supra*, 1989) upstream from the human γ1 constant (C) region using the unique EcoRI site to yield plasmid γ1NV^{2}NA^{3}. Plasmid γ1NANP carries a productively-rearranged murine V region gene in which only the CDR3 was modified by introducing the nucleotide sequence coding for three NANP repeats (Sollazzo et al., *supra*, 1990a). The promoter and enhancer elements in these plasmids are those constitutively existing in Ig H chain genes (Sollazzo et al., *supra*, 1989).